

IN THE CLAIMS

1-28 (Cancelled)

29. (Currently Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing an untransformed cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or

(C) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or

(D) culturing said cell that is sensitive to an endocrine hormone in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression pattern (4) of said cell; and

(E) comparing gene expression pattern (1) with gene expression pattern (2) and/or (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) and/or (3) and/or (4) is indicative of endocrine disrupting action by the test substance,

wherein each of the gene expression patterns (1) to (4) is measured by determining the variation in the amount of gene transcription based on an electrophoretic pattern of RNA recovered from the corresponding cultured cell or electrophoretic pattern of cDNAs produced from the RNA.

30. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2).

31. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (3).

32. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2) and gene expression pattern (3).

33. (Previously Presented) The method of Claim 29, comprising comparing gene expression pattern (1) with gene expression pattern (4).

34. (Currently Amended) The method of Claim 29, wherein said gene expression patterns are measured by determining the variation in the amount of gene transcription based on the electrophoretic patterns of cDNAs corresponding to the RNA.

35. (Previously Presented) The method of Claim 29, comprising recovering RNA corresponding to each gene expression pattern, optionally producing cDNA corresponding to said RNA, and comparing said RNA or cDNA of (A) with that of (B) and/or (C) and/or (D) to determine the endocrine disrupting activity of the test substance, wherein a difference in the amount of RNA or cDNA between (A) and (B), (C) and/or (D) is indicative of the endocrine disrupting activity of the test substance.

36. (Currently Amended) The method of Claim 29, wherein ~~the~~ RNA, or optionally the cDNA corresponding to said RNA, obtained from (A) and (B) and/or (C) and/or (D) is electrophoretically separated to determine the gene expression patterns.

37. (Currently Amended) The method of Claim 35, wherein ~~the~~ RNA, or optionally ~~the~~ cDNA corresponding to said RNA, obtained from (A) is hybridized to the RNA or cDNA obtained from (B) or (C) or (D), and the gene patterns of (A) and (B) or (C) or (D) are determined after subtraction of the hybridizing RNA or cDNA.

38. (Previously Presented) The method of Claim 29, wherein the gene expression pattern is determined by transcribed mRNA patterns, and wherein endocrine disruption is determined by identifying one or more mRNAs present in the gene expression pattern (1), but absent in at least one gene expression pattern selected from the group consisting of gene expression patterns (2), (3) and (4), or identifying one or more mRNAs absent in the gene expression pattern (1), but present in at least one gene expression pattern selected from the group consisting of the gene expression patterns (2), (3) and (4).

39. (Previously Presented) The method of Claim 29, wherein endocrine disruption is determined by identifying that a different amount of one or more types of RNA is expressed in (A), compared to (B) or (C) or (D).

40. (Previously Presented) The method of Claim 29, comprising:

- (a) recovering RNAs from (A) and (B), and/or (C) and/or (D);
- (b) subjecting the RNAs recovered in step (a) to reverse transcription;
- (c) amplifying reverse transcription products obtained in (b) by PCR; and
- (d) subjecting PCR products obtained in step (c) to electrophoresis, comparing the electrophoretic patterns of bands obtained, thereby detecting a band specific to a first gene expression pattern of (A).

41. (Currently Amended) The method of Claim 29, ~~wherein said gene expression patterns are measured by~~ further comprising determining variation in the amount of protein or glycoprotein expression between (A) and (B) and/or (C) and/or (D).

42. (Currently Amended) The method of Claim 29, ~~wherein~~ further comprising electrophoretically separating one or more protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) ~~are electrophoretically separated~~ to determine the gene expression patterns.

43. (Previously Presented) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) are electrophoretically separated using SDS-PAGE to determine the respective gene expression patterns.

44. (Previously Presented) The method of Claim 42, wherein the protein(s) or glycoprotein(s) expressed in (A) and (B) and/or (C) and/or (D) are electrophoretically separated using two-dimensional electrophoresis to determine the respective gene expression patterns.

45. (Currently Amended) The method of Claim 29, ~~wherein endocrine disruption is determined by~~ further comprising identifying that a different amount of a protein or glycoprotein is expressed in the gene expression pattern of (A), compared to (B) or (C) or (D).

46. (Currently Amended) The method of Claim 29, further comprising determining a variation in the amount of protein modification in said gene expression patterns, wherein a variation in protein modification between one or more proteins in the gene expression pattern of (A) compared to (B) and/or (C) and/or (D) is indicative of an endocrine disrupting activity of the test substance.

47. (Currently Amended) The method of Claim 46, where the variation in protein modification is measured by:

recovering the glycosylated proteins of (A), and (B) and/or (C) and/or (D) by binding them to a substance that binds to a polysaccharide chain,

cleaving the polysaccharide chain from the glycoprotein, and

determining the gene expression patterns obtained from (A) and (B) and/or (C) and/or (D) based on a comparison of the glycoproteins after cleavage.

48. (Previously Presented) The method of Claim 29, wherein said cell is a germ cell.

49. (Previously Presented) The method of Claim 29, wherein said cell is a nerve cell.

50. (Previously Presented) The method of Claim 29, wherein said cell is a normal cell.

51. (Previously Presented) The method of Claim 29, wherein said cell is a cancer cell.

52. (Previously Presented) The method of Claim 29, wherein said cell is a nonhuman mammalian cell.

53. (Previously Presented) The method of Claim 29, wherein said cell is a human cell.

54. (Currently Amended) The method of Claim 29, wherein said untransformed cell is not a genetically engineered cell.

55. (Previously Presented) The method of Claim 29, wherein said cell is selected from the group consisting of a murine neuroblastoma cell, a murine uterus carcinoma cell, a murine testicular Leydig cell, a cell derived from testicular Sertoli cells.

56. (Previously Presented) The method of Claim 29, wherein said cell is selected from the group consisting of Neuro2a, MCF7, TM3, TM4, 15P-1 and S-20Y.

57. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is a female hormone.

58. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is estrogen, estradiol, or progesterone.

59. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is a male hormone.

60. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is androgen, testosterone, or androsterone.

61. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is an adrenal cortex hormone.

62. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is cortisol, aldosterone, corticosterone or cortisone.

63. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is an amino acid derivative hormone.

64. (Previously Presented) The method of Claim 29, wherein said endocrine hormone is triiodothyronine (T3), thyroxine (T4) or a parathyroid hormone.

65. (Currently Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing an untransformed cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and/or

(C) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the absence of the endocrine hormone, but in the presence of the test substance, and detecting a gene expression pattern (3) of said cell; and/or

(D) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the absence of the endocrine hormone and in the absence of the test substance, and detecting a gene expression pattern (4) of said cell; and

(E) comparing gene expression pattern (1) with gene expression pattern (2) and/or (3) and/or (4) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) and/or (3) and/or (4) is indicative of endocrine disrupting action by the test substance.

66. (Currently Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing an untransformed cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell; and

(B) culturing said cell, which has not been genetically engineered and which is sensitive to an endocrine hormone, in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and

(C) comparing gene expression pattern (1) with gene expression pattern (2) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) is indicative of endocrine disrupting action by the test substance, and

wherein each of the gene expression patterns (1) and (2) is measured by determining the variation in the amount of gene transcription based on an electrophoretic pattern of RNA recovered from the corresponding cultured cell of electrophoretic pattern of cDNAs produced from the RNA.

67. (Currently Amended) A method of detecting an endocrine disrupting action of a test substance, comprising:

(A) culturing an untransformed cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone and the test substance and detecting a gene expression pattern (1) of said cell, and

(B) culturing said cell that is sensitive to an endocrine hormone in the presence of the endocrine hormone, but in the absence of the test substance, and detecting a gene expression pattern (2) of said cell; and

(C) comparing gene expression pattern (1) with gene expression pattern (2) to determine the endocrine disrupting activity of the test substance, wherein the increased or decreased expression of a gene in expression pattern (1) compared to the same gene in expression pattern (2) is indicative of endocrine disrupting action by the test substance.

68. (Previously Presented) The method of Claim 29, wherein gene expression pattern (1) is compared with gene expression pattern (2), gene expression pattern (3) and gene expression pattern (4).

69. (Previously Presented) The method of Claim 68, wherein gene expression pattern (1) is compared with gene expression pattern (2) to afford a comparison result, and then the comparison result is compared with the gene expression pattern (3) and gene expression pattern (4).